



Kebar Grass Extract (*Biophytum Petersianum*) Increases Diameter Of The Seminiferous Tubules Of Diabetic Mice

Faraida Arvilla¹, Reny I'tishom², Bambang Purwanto³

¹Reproductive Health Science Faculty of Medicine, Airlangga University.

²Department of Medical Biology, Faculty of Medicine, Airlangga University.

³Department of Medical Physiology, Faculty of Medicine, Airlangga University.

Email : faraida.arvilla19@gmail.com

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ABSTRACT

Diabetes is a metabolic disease that can affect the male reproductive system. The condition of hyperglycemia will increase the production of ROS, causing oxidative stress. Oxidative stress causes microangiopathy, triggers cell apoptosis, interferes with spermatogenesis, thereby affecting the diameter of the seminiferous tubules. Kebar grass contains flavonoid and vitamin E which can be used to reduce ROS reactions and inhibit oxidative stress. This research was to prove that kebar grass extract can increase diameter of the seminiferous tubules of diabetic mice. This research is a true experimental design with post test only control group design. Total of 30 samples were divided into 5 groups: negative control group (K-) was given CMC-Na, positive control group (K+) was given metformin 2mg/kg, groups P1, P2 and P3 received 2mg/kg metformin and kebar grass extract with different dosages (67.5mg/kg, 135mg/kg and 270mg/kg). The treatment lasted for 35 days. On the 36th day the mice were sacrificed and the testes were taken, then histological observations were made with HE staining to measure the diameter of tubules. The result showed that there was a significant difference in the diameter of seminiferous tubules with p value=0,000. Kebar grass extract can increase the diameter of seminiferous tubules of diabetic mice.

Keywords : diabetes mellitus, diameter of the seminiferous tubules, kebar grass.

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INTRODUCTION

Diabetes mellitus is a global health problem because it affects more than 400 million people worldwide and is expected to increase to more than 600 million by 2045 (International Diabetes Federation, 2017). Indonesia get sixth ranks in the world for the highest prevalence of diabetes mellitus after China, India, the United States, Brazil and Mexico with 10.3 million diabetics (International Diabetes Federation, 2017).

Diabetes mellitus is a chronic metabolic disorder due to the pancreas not being able to produce enough insulin or the body cannot use insulin that has been produced effectively (Kementrian kesehatan, 2014). This disease is characterized by increased glucose levels that exceed

normal (hyperglycemia) associated with long-term damage, dysfunction and failure of several organs in the patient's body (Singh et al., 2012). Clinical and experimental data indicate that diabetes adversely affects reproductive function especially in men which can cause a decrease in fertility rates (Maresch et al., 2018). Diabetes mellitus describes a group of metabolic disorders characterised by high blood glucose levels (Cho et al., 2018). Conditions of high glucose levels in the blood can cause excessive ROS formation in the body resulting in oxidative stress. One of the primary markers of excessive ROS formation is an increase in malondialdehyde (MDA). Increased levels of MDA in plasma, liver, and tissue have been reported in diabetic patients (Tiwari et al., 2013). Oxidative stress that occurs in the testes will result in damage to the mitochondrial membrane and cause loss of potential function of the mitochondrial membrane which will lead to cell death or apoptosis. Oxidative stress also causes damage to blood vessel endothelium and causes microangiopathy, which interferes with the supply of nutrients through the blood vessels to the spermatozoa-forming tissues. That will interfere the process of spermatogenesis that occurs in the seminiferous tubules (La Vignera et al., 2015).

Oxidative stress cannot be overcome by endogenous antioxidants, so they require exogenous antioxidants. Therefore, we need medicines that contain exogenous antioxidants that work on free radicals and prevent the effects of damage to the body. Flavonoids and vitamin E are one of the exogenous antioxidant groups, as radical-scavenging antioxidants that capture free radicals before free radicals attack lipids and capture peroxy lipid radicals (Yuslianti, 2018). One source of exogenous antioxidants in Indonesia is the kebar grass (*Biophytum petersianum* Klotzsch). Kebar grass is a medicinal plant that grows in Indonesia, especially in West Papua, has been used for generations by local people as traditional medicine in improving reproductive performance (Unitly & Inara, 2011). Kebar grass contains antioxidant compounds namely flavonoids and vitamin E which can stabilize free radicals by completing the lack of electrons and inhibiting oxidative stress. The entry of compounds contained in the kebar grass through the blood will reach all cells and tissues of the body including the reproductive organs, namely the testes in which there are seminiferous tubules where spermatogenesis occurs. Active antioxidants contained in kebar grass can reduce oxidative damage to cells and prevent the death of spermatogenic cells in the seminiferous tubules (Lefaan et al., 2014).

Various studies on the effects of kebar grass (*Biophytum petersianum* Klotzsch) on reproduction in animals have been carried out. Kebar grass infusion can increase spermatogenesis activity in male mice (Lefaan et al., 2014). In addition, kebar grass extract is effective for maintaining the quality of the spermatozoa of mice from damage due to exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (Kurnijasanti & Suprayogi, 2020).

Given the previous research that has been carried out, kebar grass has been shown to have a good effect on male fertility. In this study, the researchers intend to conduct research on the effects of Kebar grass (*Biophytum petersianum* Klotzsch) given to diabetes mellitus model mice.

The aim of this study is to prove the potential for administration of kebar grass extract (*Biophytum petersianum* Klotzsch) in increasing the diameter of the seminiferous tubules of diabetic mice.

METHODS

This research was true experimental design research with post test only control group design. The research was conducted at the Faculty of Medicine, Airlangga University between November 2019 to January 2020. The sample in this study was male mice from Balb/C strains aged 6-8 weeks, body weight 20-30gram.

The making of kebar grass extract is macerated with 70% ethanol solvent. Mice were made diabetic by intraperitoneally inducing streptozotocin 40mg/kg BW for 5 consecutive days. Successful induction of streptozotocin is characterized by blood sugar levels of mice > 200mg/dL.

The samples of 30 diabetic mice were divided into 5 groups: negative control group (K-), positive control group (K+) were given metformin 2mg/kg, groups P1, P2 and P3 received metformin 2mg /kg and kebar grass extract with different doses (67.5 mg/kg, 135 mg/kg and 270mg/kg). After 35 days of treatment, the mice were sacrificed and dissected for their testicular organs, then microscopic preparations were made using the paraffin method and Hematoxylin Eosin staining. The diameter of the seminiferous tubules is measured under a light microscope at 400x magnification in 10 visual fields. Statistical analysis was performed with the Anova test.

RESULTS AND DISCUSSION

The aim of this study was to prove that administration of kebar grass extract can increase the diameter of the seminiferous tubules in diabetic mice.

Table. 1 Mean and standart deviation, shapiro-wilk test, levenne test, and anova test diameter of the seminiferous tubules of diabetic mice.

Groups	Mean \pm SD	<i>Shapiro-wilk test</i>	<i>Levenne test</i>	<i>Anova</i>
				<i>p value</i>
negative control group	195.82 \pm 8.74	0.993	0.056	0.000*
positive control group	230.17 \pm 26.42	0.182		
Kebar grass 67,5 mg/kg BB	230.55 \pm 14.12	0.789		
Kebar grass 135 mg/kg BB	239.61 \pm 5.73	0.522		
Kebar grass 270 mg/kg BB	244.86 \pm 18.23	0.436		

* $p > 0,05$ = significant difference.

Table 1 showed the highest average diameter of the seminiferous tubules of diabetic mice was at dose of 270 mg/kg (244.86), while the lowest was in the negative control group (195.82).

Anova test results of seminiferous tubule diameter is $p = 0,000$ ($p < 0.05$) which means there is a significant difference in the seminiferous tubule diameter of all groups of mice. These results prove that the induction of streptozotocin causes hyperglycemia which causes oxidative stress, interfering with the formation of spermatozoa so that it can reduce the diameter of the seminiferous tubules of diabetic mice.

Research by Xu (Xu et al., 2014) states that rats injected with streptozotocin show an increased apoptosis index of Sertoli cells and decreased number of Leydig cells (Kermani et al., 2019). The same thing was also expressed by Kong et al (Kong et al., 2018), that diabetes mellitus can stop the growth and development (atrophy) of Leydig cells and Sertoli cells. Sertoli cells are somatic cells in the seminiferous tubular epithelium that provide support for the development and maturation of germ cells in the seminiferous tubules, so that the apoptosis of Sertoli cells will affect germ cells in the seminiferous tubules. Research by Bozkurt et al (Bozkurt et al., 2018) states that diabetes mellitus rats with streptozotocin induction result in oxidative stress which affects the spermatogenesis process. The same thing was also expressed by Adelati et al (Adelati et al., 2016) that the hyperglycemia condition in diabetes can interfere with the stage of spermatogenesis, causing degeneration of spermatogenic cells in the seminiferous tubules (Öztaş et al., 2019) thereby affecting the diameter of the seminiferous tubules in mice.

The hyperglycemia state causes the buildup of Advanced Glycation End Products (AGEs) and increases the formation of Reactive Oxygen Species (ROS) (Rhee & Kim, 2018). The formation of reactive oxygen compounds can increase the modification of lipids, DNA, and proteins in various tissues. Molecular modification in these tissues results in an imbalance between protective antioxidants (antioxidant defenses) and increased ROS production. ROS production is actually a physiological process of the body but if there is an imbalance between production and elimination by antioxidants in the body, accumulation of ROS in tissues can cause oxidative stress. Oxidative stress that occurs in the testes will result in damage to the mitochondrial membrane and cause loss of potential function of the mitochondrial membrane which will lead to cell death or apoptosis (La Vignera et al., 2015), causes disruption of spermatogenesis and decreased sperm quality (Aitken et al., 2014).

Diabetes mellitus causes an increase in ROS that damage the mitochondrial membrane. This causes a loss of potential function of the mitochondrial membrane and will induce apoptosis of spermatozoa. This is the same as the research of Anindita and Sutjarso (Anindita & Sutjarso, 2012) which states that the diameter of the seminiferous tubules is influenced by the spermatozoa production.

Diabetes mellitus rats get damage to the reproductive system by showing testicular dysfunctions (Fatani et al., 2015), reduction in tubular diameter and volume (Abbasi et al., 2013), irregular spermatogenic cells, inhibited spermatogenesis and vacuolization in spermatogenic cells (Oladipo et al., 2018).

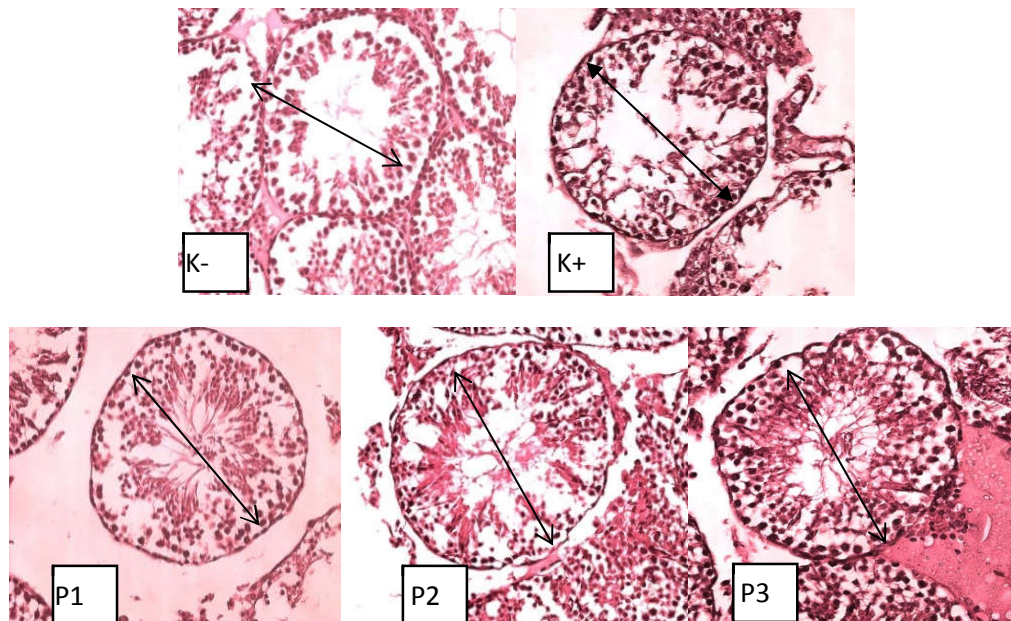


Figure 1. Histological picture of seminiferous tubules using a light microscope with 400x magnification with HE staining in the negative control group K- (CMC Na 1%), positive control group (K+) (metformin 2mg/kg), P1 group (metformin 2mg/kg + kebar grass extract dose 67.5mg/kg), P2 group (metformin 2mg/kg + kebar grass extract dose 135mg/kg), and group P3 (metformin 2mg/kg + kebar grass extract dose 270mg/kg).

Figure 1 can be seen that the group that received the kebar grass extract (*Biophytum petersianum*) showed a significant difference in the diameter of the seminiferous tubules compared to the negative control mice group. This is caused by the kebar grass has several active ingredients that act as antioxidants, namely flavonoids and vitamin E (Sembiring & Darwati, 2016). These antioxidants can eliminate free radicals and reduce oxidative stress, so the amount of ROS and antioxidants is in a balanced state so as to reduce the negative effects of oxidants (Sayuti & Rina, 2015). Antioxidant enzymes will reduce oxidative damage to cells and can inhibit apoptosis in germ cells of testicular diabetic mice (Mallick et al., 2010). Unitley (Unitley & Inara, 2011) in his research explained that the kebar grass (*Biophytum petersianum*) can increase the activity of cells that produce FSH and LH in the pituitary. This increase will trigger an increase in the production of the hormones testosterone and ABP (Androgen Binding Protein) which are used in the spermatogenesis process.

Flavonoid has a significant effect on increasing spermatogenesis activity in mice (Lefaan et al., 2014), and vitamin E plays a role in the formation of the hormone testosterone so that it can increase the process of spermatogenesis (Sembiring & Darwati, 2016). The optimal spermatogenesis process will produce good spermatozoa that will increase the diameter of the seminiferous tubules. This is same with the research of Yue et al (Yue et al., 2010), which states that vitamin E can increase the diameter of the seminiferous tubules. Vitamin E as antioxidant plays a role as a primary protector against oxidative stress and prevents the production of lipid peroxide by

free radicals. It shows that the content of kebar grass (*Biophytum petersianum* Klotzsch) can increase the diameter of the seminiferous tubules of diabetic mice.

CONCLUSION

Kebar grass extract (*Biophytum petersianum* Klotzsch) can increase the diameter of the seminiferous tubules in diabetic mice (*Mus musculus*). Similar research needs to be done in the future to see testosterone levels in diabetic mice.

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